Complementation of Coat Protein Mutants of Pepper Huasteco Geminivirus in Transgenic Tobacco Plants

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ABSTRACT

Guevara-González, R. G., Ramos, P. L., and Rivera-Bustamante, R. F. 1999. Complementation of coat protein mutants of pepper huasteco geminivirus in transgenic tobacco plants. *Phytopathology* 89:540-545.

The role of the pepper huasteco virus (PHV) coat protein (CP) gene during the infection was investigated in three different hosts by using mutations that produced truncated proteins and by complementation assays in transgenic plants. The infectivity analysis revealed that mutants that express truncated CP (CP7 and CP191) behave like the wild-type virus when inoculated onto pepper and *Nicotiana benthamiana* plants in terms of symptom expression and viral DNA movement. On the contrary, the CP7 mutant was unable to systemically infect tobacco plants, whereas

only 10% of the plants inoculated with the CP191 mutant became infected. The CP7 mutant was complemented by coinoculating it with another geminivirus (taino tomato mottle virus). No complementation was observed in plants from nine transgenic tobacco lines expressing CP under the control of the cauliflower mosaic virus (CaMV) 35S promoter. However, 3 out of 10 lines expressing CP under the control of its own promoter (693 nucleotides) were able to complement the CP7 mutant. Interestingly, upon infection, the levels of CP mRNA in 693CP plants increased dramatically, probably due to transactivation of the CP promoter by the viral protein AC2.

Additional keywords: geminivirus promoter, AC2 transactivation.

The *Geminiviridae* is a family of plant viruses with small twinned isometric particles and genomes containing one or two single-stranded DNA (ssDNA) molecules. Geminiviruses are classified into three genera according to their host range, insect vector, and genome organization. Members of the genus *Begomovirus* are transmitted by the whitefly *Bemisia tabaci* (Gennadius), infect dicotyledonous plants, and have a genome comprised of one or two ssDNA molecules (19). Pepper huasteco virus (PHV) is a bipartite begomovirus widely distributed in horticultural areas in Mexico and the southern United States, and it is probably the most important geminivirus affecting Mexican agriculture (31,32). Similar to other bipartite geminiviruses, PHV contains several open reading frames (ORFs) on each component. Component A contains ORFs involved in replication (rep and AC3), encapsidation (CP), and transcriptional activation of virion sense genes (AC2). Component B, on the other hand, harbors two ORFs involved in viral movement within the plant.

It has generally been considered that a viral systemic infection of a host plant occurs in two steps. First, the virus enters a wounded cell, and viral proteins including a nonstructural movement protein (MP) are synthesized. MP allows viral movement among cells through plasmodesmata (short-distance or cell-to-cell movement). The second step occurs when, via short-distance movement, a virus reaches the vascular system and the host plant becomes systemically infected (long-distance movement) (7,14,28). It has also been reported that several factors may influence the vascular movement of a virus throughout the host plant and, therefore, the systemic infection. Among the reported factors are the developmental stage of the host plant (16,17) and the luminosity and temperature conditions under which a host plant is incubated (35,36). In all of these ex-

amples, several viral genes have been shown to be involved in vascular movement (28). The coat protein (CP) is the viral protein to which a function in vascular movement has been most frequently ascribed.

It has been shown that CP plays an important role in vector transmission of geminiviruses (1,4). It is able to complement certain BR1 mutants (15) and, whereas it is indispensable for systemic spread in monopartite geminiviruses (5), it is not essential in the case of bipartite viruses (1,10,34). Recently, it has been reported that the dispensability of the CP for begomovirus systemic spread might be dependent on both host and viral factors (23).

In this work, the role of the PHV CP gene in the infection of several hosts was investigated. The results reported here suggest a host-dependent dispensability of PHV CP for systemic movement. Moreover, transgenic tobacco plants expressing PHV CP under its own promoter can complement CP mutants.

MATERIALS AND METHODS

Construction of PHV CP mutants. The PHV CP gene has two *SlyI* restriction sites that were used to create frameshift mutations. Mutants CP7 and CP191 were obtained by partial digestion of component A of PHV with *SlyI*, filling-in of the cohesive ends, and religation. In both cases, the frameshift would be predicted to generate truncated CPs. In the case of CP7, the resulting ORF codes for a peptide of 18 amino acids, in which only the first seven correspond to the original CP. Similarly, for CP191, an ORF coding for a polypeptide of 195 amino acids would be generated. Again, only the first 191 correspond to the wild-type (wt) CP. The presence of the mutations was verified by sequencing. A third mutant (CPΔSty) was obtained by deleting the *SlyI* fragment (556 base pairs [bp]) (Fig. 1).

Plant inoculation by a biolistic procedure. The inoculation procedure for *Capsicum annuum* cv. Sonora Anaheim, *Nicotiana benthamiana*, and *N. tabacum* Xanthi nc was carried out using a particle gun device (model PDS1000; Dupont, Wilmington DE), as reported previously (11). All mutants were excised from the plasmid vector before inoculation (3). Bombardments were done

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at a pressure of 800 pounds per square inch. Inoculated plants were maintained in a growth chamber with a light/dark photoperiod of 16/8 h for up to 8 to 10 weeks. Appearance of symptoms was scored daily, and DNA extractions were performed using the procedure described by Dellaporta et al. (8).

Polymerase chain reaction (PCR) analysis of viral progeny. To confirm the presence of viral DNA in the inoculated plant and its identity, DNA extracts from infected plants were analyzed by PCR using the oligonucleotides 142 (5' AATGCTGTACTTGA-GAATCA 3'), 143 (5' ACCTTGAATTCAAAATGCCT 3'), and 240 (5' GGCTTATTTGTAATAAGAGAGGTGT 3'). PCR set 1 included primers 240/142, which direct the amplification of a 1,062-bp fragment that extends from the common region to the 3' end of the CP ORF (Fig. 1A). Set 2 included primers 143/142, which direct the amplification of a 753-bp fragment comprising the entire CP ORF (Fig. 1A and B). The amplification products were analyzed by *Sst*I digestion/agarose gel electrophoresis and by sequencing to confirm the presence of the mutations. The PCR amplifications were carried out in a DNA thermal cycler (Perkin-Elmer Corp., Norwalk, CT) with the following conditions for both primers sets: denaturation (94°C, 1 min), annealing (50°C, 1 min), and extension (72°C, 2 min) during 30 cycles.

Hybridization analysis. The hybridization procedures (Southern and northern blots) were accomplished as described by Sambrook et al. (27). Following electrophoresis, nucleic acids were transferred onto Hybond N membranes (Amersham International plc., Little Chalfont, United Kingdom) for hybridization with DNA probes (either PHV A or tomato mottle virus [TToMoV] A DNAs) labeled with ³²P-dCTP by random priming. Northern blot analysis was done with total RNA extracted from transgenic, infected, and healthy tobacco plants as described by Verwoerd et al. (33). The exposed X-ray film was scanned, and the hybridization signals quantified with an IS-1000 Digital Imaging System (Alpha Innotech Co., San Leandro, CA).

Complementation analysis in transgenic plants. Stable transformation of *N. tabacum* Xanthi nc plants was accomplished by the

leaf disc transformation method as described by Horsch and Klee (13). For the complementation tests, the primary transformants were self-pollinated, and the seed was collected and germinated in the presence of kanamycin to select the transformed progeny.

Complementation analysis was carried out by inoculating the PHV CP7 mutant onto transgenic tobacco plants that expressed the PHV CP under the control of either its own promoter (693 bp) (26) or the cauliflower mosaic virus (CaMV) 35S promoter. After inoculation, the plants were transferred to a greenhouse for symptom development.

RESULTS

Infectivity of PHV CP mutants. To investigate the importance of PHV CP during the infection process, several CP mutations were generated (Fig. 1). Two frameshift mutations resulted in truncated CPs. Mutant CP7 contains an ORF predicted to encode a small polypeptide of 18 amino acids, of which only the first seven correspond to the wt CP. Mutant CP191 contains an ORF predicted to produce a polypeptide of 195 amino acids, of which the first 191 correspond to the CP. A third mutant, called CPΔSty, was generated by deleting the 556 bases located between the two *Sst*I sites. Similar to CP7, this deletion now contains an ORF with the capability to produce a chimeric peptide 14 amino acids long, in which the first seven correspond to the wt protein. However, in this case, the size of component A was reduced from 2,632 to 2,076 nucleotides.

The infectivity of the mutants was evaluated by inoculating three different species by a biolistic procedure: pepper (*C. annuum* cv. Sonora Anaheim), a natural host for PHV; *N. benthamiana*, a widely used experimental host; and tobacco (*N. tabacum* Xanthi nc), an atypical host for PHV. This classification as an atypical host refers to the fact that tobacco can be efficiently inoculated by biolistics, developing symptoms 8 to 12 days postinoculation (dpi). However, when similar plants were inoculated by grafting, the symptoms of the infected plants were visible only after 30 to 40 dpi. Table 1 summarizes the results of the infectivity tests. Infectivity was evaluated by the appearance of systemic symptoms and the presence of viral DNA in newly developed leaves. wt PHV induced systemic symptoms in pepper and *N. benthamiana* plants 7 to 8 dpi. Tobacco plants inoculated with wt PHV initially developed some chlorotic lesions at the site of inoculation. These local lesions were clearly visible 7 dpi. In a later stage (10 to 12 dpi), chlorotic lesions appeared throughout the plant. Symptoms were more severe in the apex where rugosity, reduced size, and vein deformation of leaves were typically observed. The infectivity of mutants CP7 and CP191 in pepper and *N. benthamiana* were similar to the wt virus in terms of efficiency of inoculation and appearance and severity of symptoms. However, a delay of 2 to 3 days in symptom appearance was noticed in some plants inoculated with either mutant. In the case of tobacco, CP7 was not able to produce a systemic infection, generating only the typical chlorotic local lesions on the inoculated leaves. Only a low percentage (10%) of the plants inoculated with CP191 developed a systemic infection. These plants, however, showed a delay of 2 months for symptom development. Once the symptoms were fully expressed, their severity was similar to those induced by the wt virus.

Fig. 1. Pepper huasteco virus (PHV) genomic organization and coat protein (CP) mutants. **A**, PHV genomic organization and location of selected restriction sites. The location of the primers used for polymerase chain reaction amplification of the CP gene is also shown. The fragment used as the CP promoter in 693CP plants extends from the open reading frame (ORF) to the *Eco*RI site. **B**, Predicted ORFs in wild-type PHV and in the different CP mutants. The location of the *Sst*I sites used to create CP7, CP191, and CPΔSty is also shown.

TABLE 1. Systemic infectivity of pepper huasteco virus (PHV) mutants^a

PHV	Pepper	<i>Nicotiana benthamiana</i>	Tobacco
Wild type	20/21	21/23	23/25
CP7	18/20	22/25	0/50
CP191	15/17	21/25	2/20 ^b
CPΔSty	15/40	ND ^c	ND

^a Systemic infectivity was evaluated by the expression of systemic symptoms and the presence of viral DNA on newly developed leaves. Number of systemically infected plants/number of inoculated plants.

^b Symptom development in these plants was delayed approximately 2 months.

^c Not determined.

The presence of viral DNA was evaluated in the inoculated plants by Southern blot hybridization and PCR amplification. Figure 2 shows that, indeed, CP7 and CP191 mutants can efficiently move systemically in pepper and *N. benthamiana* (lanes 2 and 3 and 8 and 9). The amount of viral DNA recovered from CP7- or CP191-inoculated pepper or *N. benthamiana* plants was lower than the amount recovered from plants inoculated with wt PHV (lanes 1 to 3 and 7 to 9). No viral DNA was detected in tobacco plants inoculated with CP7 or CP191 21 days after inoculation (lanes 5 and 6). Only two of the CP191-inoculated tobacco plants developed symptoms 60 dpi. In these plants, viral DNA could be recovered from the young symptomatic leaves only after the symptoms were expressed. Again, the amount of viral DNA was reduced when compared with the wt control. The viral progeny extracted from all plants inoculated with CP7 and CP191 was analyzed by PCR amplification and sequencing and by digestion with *StyI*. The analysis confirmed the presence of the mutations and no revertant wt virus was detected in any case (data not shown).

The failure of PHV CP7 to produce a systemic infection in tobacco plants could be due either to a lack of virus replication or to defective virus movement. To differentiate between these two possibilities, DNA from CP7-inoculated tobacco leaves was obtained and analyzed by Southern blot for the presence of viral replicative forms (RF). The analysis showed that, indeed, viral RF could be detected in those leaves, demonstrating that replication of the CP7 mutant is not impaired in tobacco cells (data not shown).

Similar infectivity assays were performed with the CPΔ*StyI* mutant. In this case, the virus mutant was able to systemically infect pepper, despite its smaller genomic size (Table 1). However, pepper plants inoculated with this mutant showed variable symptom expression that included asymptomatic plants and different degrees of attenuated symptoms. The appearance of the mild symptoms was usually delayed 2 to 3 weeks. In all cases, the concentration of viral DNA detected in the newly developed leaves of the inoculated plants was lower than the levels detected in wt-inoculated pepper plants (data not shown).

Complementation analysis of the PHV CP7 mutant. Recently, a tomato-infecting geminivirus was cloned from samples collected in Cuba and tentatively named TToMoV (24). Since the CPs of PHV and TToMoV are 88% identical at the amino acid level, an attempt to complement the PHV CP7 mutant with TToMoV was carried out by coinoculating both viruses into tobacco plants, which is also a host for TToMoV. Ten days after inoculation, the inoculated plants developed symptoms, and DNA from the newly developed tissue was extracted and analyzed for the presence of both viruses by Southern blots using virus-specific probes.

Fig. 2. Detection of viral DNA in wild-type-, CP7-, and CP191-infected plants. Southern hybridization of DNA extracts from pepper (lanes 1 to 3), tobacco (lanes 4 to 6), and *Nicotiana benthamiana* (lanes 7 to 9) plants inoculated with wild-type pepper huasteco virus (PHV) (lanes 1, 4, and 7), CP7 (lanes 2, 5, and 8), or CP191 (lanes 3, 6, and 9) DNA. DNA was extracted from the young newly developed leaves 21 days postinoculation (dpi) (in pepper and *N. benthamiana* symptoms appeared 7 to 8 dpi). ³²P-labeled PHV A DNA was used as a probe.

In contrast with the result observed in CP7-inoculated tobacco (Fig. 2, line 5), both TToMoV and CP7 DNAs were easily detected in the coinoculated plants (Fig. 3, lanes 1 and 5), suggesting that TToMoV was able to facilitate the movement of PHV CP7. Again, PCR and digestion with *StyI* confirmed the identity of the CP7 mutant.

The complementation of CP7 by TToMoV could be the result of a more complex synergistic interaction involving several factors. Thus, another strategy, in which transgenic tobacco plants expressing the PHV CP gene were produced, was followed. Two types of tobacco transgenic plants were generated. In the first set, nine independent transgenic lines in which the CP gene was under the control of the CaMV 35S promoter were inoculated with the CP7 mutant. None of the plants developed systemic symptoms, and no viral DNA was detected in the newly developed tissue in those plants (Table 2). Northern blot analysis demonstrated that the overall levels of CP mRNA in total RNA extracts from leaf tissue were generally high (Fig. 4, lanes 3 and 4). However, it was possible that the level of CP in the required tissues (i.e., vascular tissue) was not sufficient to complement the movement of PHV CP7. Therefore, another set of transgenic tobaccos were obtained in which the CP ORF was under the control of its own native promoter (693 nucleotides upstream from the initiation codon) (Fig. 1). The expression of this promoter (693CP) has been characterized in transgenic tobacco and potato plants by GUS fusions (26; R. Ruiz-Medrano and R. Rivera-Bustamante, *unpublished data*), and it has shown to be very strong in vascular tissue. Ten independent transgenic lines were evaluated by inoculating them with the CP7 mutant. In seven cases, no symptoms were observed up to 60 dpi, and no viral DNA was detected in the newly developed tissue of the inoculated plants. However, several plants from lines 5, 6, and 11 became systemically infected (Table 2). The appearance of the symptoms was delayed 8 to 10 days when compared with the control of nontransgenic tobacco inoculated with the wt virus. In addition, the symptoms were initially milder and associated with the vascular tissue (yellowing spots near the primary and secondary leaf veins). However, in a later stage, the symptoms became almost indistinguishable from symptoms observed in the wt control. There was no correlation between insert copy number in the transgenic lines and their ability to complement the CP7 mutant. As an additional control, several plants from all transgenic lines of each construct (35SCP and 693CP) were inoculated with wt PHV. No difference was observed between the transgenic and the nontransgenic tobaccos in terms of inoculation efficiency (percentage of infected plants) and appearance and severity of the symptoms. This suggests that the expression of PHV CP in tobacco did not induce any resistance.

Northern analysis of all the 693CP lines showed that the levels of CP mRNA were very low in the uninoculated transgenic plants. However, upon inoculation, the infected plants showed a very

Fig. 3. Complementation of CP7 by taino tomato mottle virus (TToMoV) in mixed infections. Tobacco plants were inoculated with CP7 + TToMoV (lanes 1 and 5) or wild-type pepper huasteco virus (PHV) + TToMoV (lanes 2 and 6). Young tissue was collected on the onset of the symptoms (8 to 10 days postinoculation), and DNA was extracted and hybridized with specific probes for TToMoV (lanes 1 to 4) or PHV (lanes 5 to 8). As hybridization controls, DNA extracts from TToMoV- (lanes 3 and 7) or PHV-infected (lanes 4 and 8) plants were included. The membrane for lanes 5 to 8 is actually the same one shown in lanes 1 to 4 after regeneration and rehybridization with the PHV-specific probe. Arrowheads show the position of main viral DNA forms (top, circular double-stranded DNA; bottom, circular single-stranded DNA).

strong expression of CP mRNA (Fig. 4, lane 9). This increase in expression could not be explained just by the presence of the viral mRNA, since in nontransgenic wt-infected plants, the mRNA levels were also low (Fig. 4, lane 2). The most likely explanation for the increase of CP expression is a process of transactivation of the CP transgene by viral factors (e.g., AC2) as reported in tomato golden mosaic virus (29,30).

DISCUSSION

CP is not essential for PHV systemic infection of permissive hosts. The movement of a virus in an infected plant is generally divided into two types: local or cell-to-cell movement (via plasmodesmata) and long-distance movement (via phloem sieve elements) (18). For geminiviruses, systemic movement has been suggested to have three components: (i) CP-independent cell-to-cell movement; (ii) CP-dependent systemic movement; and (iii) CP-independent systemic movement in specific hosts (23). The last component will be important in the case of a highly compatible interaction between a virus and a specific host. In the case of PHV, originally isolated and cloned from peppers, recent surveys have shown that, indeed, pepper is the main host for PHV, although some variants can now be found in other hosts (e.g., tomato) (31). Infectivity studies with accessions from pepper germ plasm banks that included several *Capsicum* species showed that PHV is well adapted to the *Capsicum* genetic background (I. Torres-Pacheco and R. F. Rivera-Bustamante, unpublished data).

The infectivity assays carried out with the CP mutants demonstrated that the CP is not essential for a systemic infection in pepper and *N. benthamiana*. The CP7 mutant, which produces an extremely truncated version of the CP, was able to induce systemic symptoms in both hosts. The severity of the symptoms was similar to those induced by the wt virus. However, minor differences (e.g., delay of 2 to 3 days in symptom appearance and lower amount of viral DNA recovered) were noticed in some cases. This suggests that, although PHV can move systemically in the absence of CP in pepper and *N. benthamiana*, the movement could be slightly more efficient when the CP is present. Similar results were obtained with the CP191 mutant, which is expected to produce a larger but still truncated CP. Finally, the CPAS_{ty} mutant, in

which most of the CP gene has been deleted, was also able to move systemically. Major differences in this case were the lower inoculation efficiency, the variability of the symptoms induced, and the amount of viral DNA present in the infected tissue. However, most of these effects could be attributed to the smaller size of the PHV A molecule, since the size seems to be important for recognition by the viral MP (25). Similar results have been observed with other geminiviruses (1,9,10).

It was not surprising that PHV was able to move systemically in the absence of CP, since similar results have been reported for other geminiviruses (23). Nevertheless, nothing is known about the mechanism for CP-independent movement. The results with *N. benthamiana* might be partially explained as a result of the high permissiveness of this host to the infection of several viruses (6). It has been suggested that this permissiveness might be due to the fact that the plasmodesmata found in *N. benthamiana* have a larger size exclusion limit (SEL) than those found in other species (B. Lucas, personal communication). However, in addition to the larger SEL, the recognition between the virus and the host is also important. For example, Petty et al. (22) recently reported that bean golden mosaic virus shows a high adaptation to beans but a poor adaptation to *N. benthamiana*. In any case, it has been suggested that some of the results obtained with this plant could be difficult to extrapolate to other species (7,12).

CP-independent systemic movement of PHV is inefficient in tobacco. The lack of efficient systemic movement of CP mutants in tobacco suggests that the virus relies mostly on CP-dependent movement for its systemic dispersion in this host. CP-independent cell-to-cell movement in tobacco lesions is probably too slow to efficiently produce a systemic infection, indicating that PHV is not well adapted to this species. The delayed symptom expression of the CP191-infected plants seems to support this hypothesis. The expected truncated CP still contains the putative zinc finger domain conserved among CPs of whitefly-transmitted geminiviruses (21). It is possible that this truncated CP was still able to partially function and allow the viral DNA to move out of the inoculated leaves.

PHV CP mutants can be complemented in transgenic tobacco plants. Several reports have suggested that some geminiviruses are restricted to the vascular tissue. In the case of PHV, tissue printing experiments have also suggested the localization of viral DNA mainly in vascular tissue (R. G. Guevara-González and R. F. Rivera-Bustamante, unpublished data). However the factors involved in this restriction have not been determined in any case.

TABLE 2. Complementation of pepper huasteco virus CP7 mutant in transgenic tobacco plants

Inoculated plants	Infected plants/inoculated plants ^a
Nontransgenic tobacco	0/30
35SCP transgenic tobacco	0/257 (all lines)
Line 1	0/28
Line 2	0/26
Line 3	0/34
Line 4	0/28
Line 5	0/33
Line 6	0/32
Line 7	0/28
Line 8	0/29
Line 9	0/19
693CP transgenic tobacco	40/198 (all lines)
Line 2	0/17
Line 4	0/19
Line 5	15/25
Line 6	18/26
Line 7	0/23
Line 8	0/21
Line 10	0/17
Line 11	7/12
Line 14	0/21
Line 16	0/17

^a Number represents all plants inoculated in three independent experiments. Infectivity was evaluated by symptom appearance and polymerase chain reaction detection of viral DNA.

Fig. 4. Northern blot analysis of 35SCP and 693CP transgenic tobacco lines. Total RNA was extracted from different control and transgenic tobacco lines, and the levels of coat protein (CP) mRNA were analyzed in northern blots. Fifteen micrograms of total RNA was loaded in each lane. Lane 1, Uninoculated nontransgenic tobacco; lane 2, pepper huasteco virus (PHV)-infected nontransgenic tobacco; lanes 3 and 4, two uninoculated 35SCP lines; lanes 5 to 7, three uninoculated 693CP lines; lane 8, uninoculated 693CP (line 6); and lane 9, PHV-infected 693CP (line 6). Upper panel: A 556-bp fragment probe from the CP gene. Lower panel: To verify RNA load, the membrane from the upper panel was regenerated and hybridized with a probe from human 18S ribosomal DNA.

The complementation of CP7 by TToMoV suggests that both viruses might be localized to the same type of cells. On the other hand, it was interesting that CP7 movement followed the pattern observed with TToMoV (e.g., rapid symptom expression), suggesting that CP7 was being "driven" by TToMoV. This type of interaction could be beneficial for the dispersal of some viruses by extending their host range to other species initially not important as primary hosts.

The CaMV 35S promoter has been reported as a constitutive promoter that is expressed in most plant tissues (2). More recently, however, it has been shown that it can also produce a mosaic-like expression pattern (20). None of the 35SCP transgenic lines produced here was able to complement the systemic movement of the CP7 mutant, even though their overall expression of CP mRNA was high. A possible explanation was that the CP was not being expressed in the proper type of tissues or in the required concentration. Similar results have been observed with squash leaf curl virus, in which transgenic tobacco plants expressing BC1 or BV1 genes under the control of the CaMV 35S promoter are not able to complement the BC1/BV1 mutants (S. Lazarowitz, *personal communication*).

The expression pattern of several PHV promoters have been studied by GUS fusions (R. Ruiz-Medrano and R. F. Rivera-Bustamante, *unpublished data*). The 693CP promoter has been shown to direct a strong but specific expression localized to the vascular and meristematic tissues. The delay in symptom expression observed in the 693CP plants inoculated with CP7 suggests that CP7 movement is still not as efficient as the wt virus in normal plants. It is not clear if this delay is a problem of the amount of CP or to its temporal/spatial localization. Transgenic and transient experiments have demonstrated that the CP promoter used here is able to be transactivated by AC2 (26). The increased levels of CP mRNA in the infected 693CP plants suggests that viral factors (i.e., AC2) can indeed transactivate the transgene (29,30). However, the transgene might not have the same overall regulation as its viral counterpart, and this outbalanced regulation might be affecting the virus cycle.

The complementation observed here could be the result of a combination of several characteristics of the geminiviral promoter (e.g., tissue specificity and transactivation by viral proteins) not found in the 35S promoter. Therefore, this type of promoter could be an interesting alternative for specific uses in crop protection.

ACKNOWLEDGMENTS

This research was supported by a grant from CONACYT-México (4769-N9406) and ICGEB (CRP/MEX94-03; 95/005) to R. F. Rivera-Bustamante. R. G. Guevara-González received fellowship support from CONACYT. P. L. Ramos received support from the BETCEN program (UNESCO) with a Training Fellowship. We thank R. Ma. Rangel-Cano and J. T. Ascencio-Ibañez for excellent technical assistance, J. Simpson for critical reading of the manuscript, and Hoechst Marion Roussel Laboratories for the gift of the antibiotic Claforan.

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